EPORT DATE	3. REPORT TYPE AND DATES COVERED
09/15/93	Final Technical 07/01/90-06/30/93

4. TITLE AND SUBTITLE
Extrathalamic Modulation of Cortical Function

5. FUNDING NUMBERS
AFOSR-90-0325
6 11 02 F
2312
BS

(2)

6. AUTHOR(S)
Foote, Stephen Leroy
Pineda, Jaime A.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, San Diego
Dept. of Psychiatry, 0603
9500 Gilman Dr.
La Jolla, CA 92093-0603

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- [illegible]

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
Department of The Air Force
Bolling Air Force Base, D.C. 20332-6448
Dr Haddad

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The overall goal of the proposed studies is to characterize the effects of noradrenergic (NA) afferents on cortical information processing. Our previous studies indicate that the primate locus coeruleus (LC) system, originating in the pontine brainstem, innervates neocortex more densely than previously thought, exhibiting highly specific patterns in terms of the regional and laminar distribution of its axons across the neocortex. Previous neurophysiological observations suggest that this highly divergent system imposes state-related modulatory effects on thalamo-cortical and cortico-cortical systems. For example, we have shown that primate LC-NA neurons are more active during waking than sleep and exhibit bursts of activity during increases in attentiveness. We have also previously demonstrated that the microiontophoretic application of NA to monkey auditory cortex neurons increases the selectiveness of their responses to auditory stimuli.

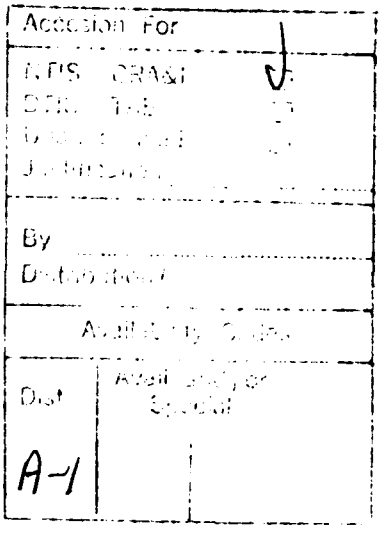
14. SUBJECT TERMS	15. NUMBER OF PAGES
	10 (ten)
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(u)	(u)	Unclassified	(u)

In studies conducted during the initial funding period, we have demonstrated that LC lesions in monkeys interfere with the production of a surface-positive wave that mimics many of the characteristics of the human P300 event-related potential (ERP). We have also found that LC activation, induced by microinfusion of drugs into the LC, produces electroencephalographic (EEG) signs of arousal in both neocortex and hippocampus. Specifically, the cortical EEG exhibits high frequency, low amplitude activity while the hippocampal EEG is dominated by theta rhythm. In addition, we have developed further assays for potential LC effects on forebrain function and we have refined our techniques for manipulating LC electrophysiological activity.

The proposed studies have the following Specific Aims: 1) To examine, in monkeys, the effects of manipulating the LC-NA system on ERPs, EEG characteristics, and associated behaviors in operant paradigms that utilize visual or auditory cues; 2) To correlate the activities of individual monkey LC-NA neurons with cortical neuronal activity and the same measures utilized in Aim 1; 3) To reproduce and extend our preliminary observation that activation of the LC by local drug infusion, in halothane-anesthetized rats, produces EEG signs of cortical and hippocampal activation; 4) To examine the relationship between the intensity of LC neuronal activity and rates of norepinephrine release in neocortex and hippocampus by performing microdialysis in these forebrain terminal regions in anesthetized rats during manipulation of LC activity.

These convergent experiments are necessary to rigorously determine whether LC-NA activity is necessary and/or sufficient for the full expression of particular aspects of cortical information processing.



FINAL TECHNICAL REPORT
(01 Jul 90 - 30 Jun 93)

CONTRACT: AFOSR-90-0325 (Foote/Pineda/Berry)

TITLE: Extrathalamic Modulation of Cortical Function

PRINCIPAL INVESTIGATORS: Stephen L. Foote/Jaime A. Pineda

DATE: 15 September, 1993

Organization of Technical Report. This report, which is comprehensive for the current 3-year funding period, is divided into four sections that correspond to the Specific Aims for Years 04-06, with an additional section for studies relevant to the overall aims of the project but not previously specifically proposed. The full-length, submitted and published reports describing studies completed during Years 04-06 are listed at the end of the Technical Report. The numbers in square brackets within the text of the report refer to the corresponding publications from this list.

AIM 1: TO EXAMINE, IN MONKEYS, THE EFFECTS OF MANIPULATING THE LC-NA SYSTEM ON EVENT-RELATED POTENTIALS (ERPs), ELECTROENCEPHALOGRAPHIC (EEG) CHARACTERISTICS, AND ASSOCIATED BEHAVIORS IN OPERANT PARADIGMS THAT UTILIZE VISUAL OR AUDITORY CUES.

The effects of systemically administered adrenergic agents on monkey P300s [Publications 1-4]. In previous funding periods we have characterized and studied the neural substrates of a monkey event-related potential component that exhibits many of the characteristics of the P3 or P300 components of human event-related potentials. The studies to be summarized in this section have evaluated the role of NA in the modulation of these auditory and visual P300 responses.

In one study [1,3], EEG, behavioral, and event-related potential data were collected from squirrel monkeys in an auditory "oddball" paradigm in which the subjects could bar-press for a food reward only during a short interval following the occurrence of target stimuli that were embedded within repetitively occurring non-target stimuli. Data were obtained following the systemic administration of placebo or clonidine, an α_2-noradrenergic agonist that suppresses LC activity and NA release. Clonidine significantly decreased the area and increased the latency of the P300-like potential that occurred following target stimuli, while leaving earlier peaks unaffected. Rates of behavioral responding were not diminished following clonidine administration. This indicates that the suppression of the P300-like potential was not due to sedation.

In the remaining studies [2,4] of this type, administration of other NA agents has been shown to alter the characteristics of these event-related potential components.

The effects of locally administered adrenergic agents on monkey P300. Systemically administered adrenergic agents can have effects at a variety of peripheral and central sites. To control for these nonspecific effects, we are currently delivering small doses of L657,743 (an α_2-antagonist) into specific

cortical sites while subjects are presented with auditory or visual "oddball" paradigms. Preliminary results show that the bilateral P3 commonly recorded in these paradigms can be unilaterally affected with drug injections into the parietal cortex of one hemisphere. With such infusions, the drug is presumably enhancing the local release of NA from endogenous stores and thus constitutes a more selective manipulation of NA neurotransmission than exogenously applied agonists.

Active and Passive Processing of Faces [Publication 5]. This study, and the remaining work completed for this Specific Aim, involve the development of electrophysiological assays of specific cortical functions that will constitute dependent variables for future studies of NA modulatory mechanisms. For example, faces are special, perhaps unique, stimuli for primates and appear to be analyzed by highly evolved cortical processing mechanisms. In order to search for electrophysiological correlates of this complex processing, these studies examined event-related potential responses to face stimuli during passive and active conditions in one juvenile and 2 young adult cynomolgus (Macaca fascicularis) monkeys. In all experiments, monkeys exhibited a P1-N1-P2 complex of peaks in the 400 ms following stimulus presentation. However, only upright (vs. inverted) faces elicited a prominent, widely-distributed negativity (N4) in the 400-800 ms interval, as well as a long-duration negativity in the last 400 ms of the 1400 ms epoch. N4 was earlier in latency, larger in amplitude, and larger over left hemisphere sites in response to monkey faces than to human faces. Trained monkey waveforms exhibited an N2-P3 complex with P3 larger in amplitude and area, delayed in latency, and slightly larger over right hemisphere in response to monkey faces. Thus, N4 appears to be sensitive to conspecific faces and is dominant over left hemisphere. Active processing results in P3-like components that may reflect the novelty and/or meaningfulness of the stimulus.

Pilot study: the relationship between single unit and P300 activity in parietal cortex. Parietal cortex appears to play a crucial role in P300 electrogenesis. We are currently correlating the activity of cells in specific areas of the parietal cortex with cortical P300 responses to auditory "oddball" events.

Pilot study: similarities and differences between human and monkey face processing. To develop a specific database of human ERPs for comparison with monkey responses to faces, we are currently recording from human subjects in paradigms similar to those used with the non-human primates (e. g., the upright and inverted faces paradigm). Additionally, we have begun a study to address the configural/feature distinction in face processing. This will address the issue of hemispheric dominance for configural processing and left hemisphere dominance for feature processing.

Publication of previously completed studies: brainstem auditory evoked potentials; intensity-amplitude relationships [Publications 6,7]. Two studies for which the work was completed during the initial funding period have now been published. Both of these studies established normative characteristics in monkey for event-related potentials that have been well studied in human.

AIM 2: TO CORRELATE THE ACTIVITIES OF INDIVIDUAL MONKEY LC-NA NEURONS WITH CORTICAL NEURONAL ACTIVITY, ERPs, EEG CHARACTERISTICS, AND ASSOCIATED BEHAVIORS IN OPERANT PARADIGMS THAT UTILIZE VISUAL OR AUDITORY CUES.

LC neuronal activity in awake monkeys: relationship to spontaneous EEG and auditory P300-like potentials [Publication 8]. These experiments were designed

to test the hypothesis that novel auditory stimuli lead to phasic and/or tonic increases in LC discharge activity, which may be a necessary condition for the occurrence of P300 potentials. Event-related potentials and LC unit activity were recorded simultaneously in 3 untrained macaque (Macaca fascicularis) monkeys during the presentation of an auditory oddball paradigm. Oddball stimuli resulted in probability-sensitive, P300-like potentials. While these event-related potential findings are novel, we were able to obtain only a limited number of high-quality LC recordings in this paradigm. Three of 12 LC units showed small phasic enhancements of LC firing after infrequent but not frequent tones. In an additional set of studies, one monkey was trained to bar-press in response to the occurrence of the target stimulus in the oddball paradigm. Interestingly, this animal displayed a prominent P300-like wave, but only when he performed the oddball task accurately. In sessions where the monkey did not respond, neither P300-like potentials nor phasic LC responses were elicited by target stimuli. However, LC cells did tend under these conditions to show a tonic elevation in firing following targets. For the 2 LC neurons whose activity we were able to record during sustained, accurate performance of the task, phasic discharge activity was observed following the presentation of targets, and the timing of this activity indicated it was related to the behavioral response rather than to stimulus presentation. Finally, comparisons of the discharge activity of individual LC neurons with EEG recordings, under circumstances where no stimuli were presented to these subjects, confirmed our previous observations in squirrel monkey that LC discharge activity is strictly correlated with, and anticipates by 500 to 1000 msec, changes in cortical EEG. Overall, our progress on this project has been limited. In addition, the graduate student (Diane Swick) who was doing this work has graduated and left the laboratory. Thus, we have decided to terminate this project.

AIM 3: TO REPRODUCE AND EXTEND OUR PRELIMINARY OBSERVATION THAT ACTIVATION OF THE LC BY LOCAL DRUG INFUSION, IN HALOTHANE-ANESTHETIZED RATS, PRODUCES EEG SIGNS OF CORTICAL AND HIPPOCAMPAL ACTIVATION.

During Years 04-06, the effects of LC activation and inactivation on neocortical and hippocampal EEG were examined in detail in anesthetized rat. All of the proposed studies were completed, and additional experiments were performed to generate pilot data for the proposed Specific Aims of the present application.

Effects of LC activation on neocortical and hippocampal EEG activity [Publications 9,10]. We have previously described a method for the selective, acute, potent, and verifiable activation of LC (3). This technique utilizes electrophysiological recordings to accurately place infusions of drugs that alter LC neuronal discharge rates. The close proximity of the infusion site allows the use of small volumes, limiting the spread of the infusion to other brainstem structures while at the same time not damaging LC. The microelectrode recordings obtained before and after the infusion provide quantitative verification of the LC manipulation and permit analyses of temporal relationships between onset and offset of LC activation/inactivation and any observed physiological effects.

In our previous studies, it was demonstrated that 50-150 nl infusions of the cholinergic agonist, bethanechol (1 ng/nl) increase LC neuronal discharge activity 3-5 times above basal levels whereas infusions of the NA α_2-agonist, clonidine (1 ng/nl) suppress LC activity. In the first series of experiments performed during this funding period, we examined whether peri-LC bethanechol-induced EEG activation is dependent on enhancement of LC neuronal discharge rates

and whether this effect could be blocked by antagonizing NA neurotransmission.

In a total of 39 animals, the findings were: 1) LC activation was consistently followed, within 5-30 sec, by a shift from low-frequency, high-amplitude to high-frequency, low-amplitude activity in the cortical EEG and the appearance of intense theta activity in hippocampus; 2) these EEG responses followed LC activation with similar latencies whether infusions were made lateral or medial to the LC; 3) infusions placed greater than 500-600 um distant from the LC were not followed by these EEG responses; 4) following infusion-induced activation, EEG returned to preinfusion patterns with about the same time course as the recovery of LC activity; 5) the infusion-induced changes in EEG were blocked or severely attenuated by pretreatment with the α_2-agonist clonidine (50 ug/kg, iv) or the β-antagonist propranolol (200 ug, icv). These observations indicate that enhanced LC discharge activity is the crucial mediating event for the infusion-induced changes in forebrain EEG activity observed under these conditions.

Effects of LC inactivation on neocortical and hippocampal EEG activity [Publication 11]. Intrabrainstem administration of α_2-agonists into the region of the LC has been observed to increase behavioral and EEG measures of sedation (4,5). Because these drugs act to inhibit LC neuronal discharge activity and NA release (6), these observations are consistent with an action of the LC/NA system in the maintenance of an activated forebrain. However, interpretation of results obtained utilizing intratissue drug infusions for the study of LC function is complicated by a variety of factors, such as the small size of the nucleus and the close proximity of the LC to other nuclei known to affect behavioral and EEG states (7,8). These factors, together with the absence of electrophysiological measures documenting the relationship between changes in LC neuronal activity and EEG state following such infusions preclude specific conclusions regarding the site(s) of action for the sedative effects of intrabrainstem administered α_2-agonists.

If, in fact, intrabrainstem administered α_2-agonists enhance EEG measures of sedation through an inhibition of LC neuronal discharge activity, it would be hypothesized that: 1) infusions that are effective in suppressing LC activity will alter forebrain EEG; 2) changes in LC neuronal discharge activity will precede changes in forebrain EEG activity; 3) the return of EEG activity to the pre-infusion state will follow the recovery of LC neuronal activity; 4) infusions that are not effective at suppressing LC neuronal discharge activity will not alter forebrain EEG measures.

In these studies, clonidine infusions (35 nl or 150 nl) were made immediately adjacent or approximately 1000 um distant to LC. These infusions were made under conditions in which high-frequency, low-voltage activity predominated in neocortical EEG and theta-activity predominated in hippocampal EEG. The following was observed: 1) cortical and hippocampal activity were not substantially affected following unilateral clonidine-induced LC inactivation; 2) bilateral clonidine infusions that completely suppressed LC neuronal discharge activity in both hemispheres induced a shift in cortical activity to low-frequency, large amplitude activity and the replacement of theta-activity with mixed frequency activity in hippocampus; 3) 35 nl infusions placed 800-1200 um from the LC did not induce a complete suppression of LC activity and did not alter forebrain EEG; 4) 150 nl infusions placed 800-1200 um from LC were either ineffective at completely suppressing LC neuronal discharge activity or did so with a longer latency to complete LC inhibition and a shorter duration of

inhibition; 5) in all cases, the latencies of EEG responses were coincident with the complete bilateral inhibition of LC discharge activity and persisted throughout the period during which bilateral LC neuronal discharge activity was completely absent (60-240 min); 6) the resumption of pre-infusion EEG activity patterns closely followed the recovery of LC neuronal activity or could be induced with systemic administration of the α_2-NA antagonist, idazoxan. These results suggest that the clonidine-induced changes in EEG were dependent on the complete bilateral suppression of LC discharge activity and that, under the present experimental conditions the LC/NA system exerts a potent and tonic activating influence on forebrain EEG state such that activity within this system is necessary for the maintenance of an activated forebrain EEG state.

AIM 4: TO EXAMINE THE RELATIONSHIP BETWEEN THE INTENSITY OF LC NEURONAL ACTIVITY AND RATES OF NORADRENALINE (NA) RELEASE IN NEOCORTEX AND HIPPOCAMPUS BY PERFORMING MICRODIALYSIS IN THESE FOREBRAIN TERMINAL REGIONS IN ANESTHETIZED RAT DURING MANIPULATION OF LC ACTIVITY.

In these experiments, peri-LC bethanechol infusions were made to increase LC neuronal discharge levels in halothane-anesthetized rats that had dialysis probes implanted in frontal neocortex. NA was assayed using HPLC with electrochemical detection. In 12 cases, rats were implanted with dialysis probes 2-3 hours prior to initiation of baseline sample collection. This procedure yielded stable baseline NA levels throughout the experiment which was conducted over the next few hours. In 6 additional cases, rats were implanted with dialysis probes the day prior to the experimental session. This was done because there is evidence that during the first 3-8 hours following dialysis probe insertion, a significant fraction of NA release is impulse independent. In the latter cases, rats were anesthetized with halothane, the dialysis probes implanted, and the rats replaced in their home cages. The following day, the rats were anesthetized with halothane, the LC located, and the experiment conducted exactly as the other 12. In all cases, 2-3 hours following initiation of halothane anesthesia, 3-4 20-min baseline samples were collected. At this point, a bethanechol infusion (1-8 ng/nl) was made at the start of a dialysis sampling interval. At the end of this 20-min sample, a recovery sample was collected, followed by a sample during which the LC was again activated, followed by 1-2 recovery samples. Infusions that increased LC neuronal discharge levels to a maximum of approximately 3 times basal levels, with a total duration of activation of approximately 10 min, resulted in a 50-100% increase in NA in dialysate samples. NA concentrations returned to baseline levels in the sample immediately following LC activation, and comparable NA responses were consistently observed with repeated LC activation. Bethanechol infusions that increased LC neuronal discharge levels to a maximum of approximately 5-6 times basal levels, with a total duration of activation of approximately 15-20 min, also increased NA concentrations 50-100%. Thus, there appears to be a ceiling beyond which increased LC discharge does not result in a corresponding increase in NA release. There are at least 2 possible explanations for this ceiling effect. First, it could result from a rapid depletion of releasable NA during the first few minutes of LC stimulation, with a subsequent recovery over approximately the next 15 min. If this is the case, it would be predicted that the first 10 min of the 20-min sample would contain substantially more NA than the 2nd 10 min, and the 2nd 10 min could contain substantially less NA than preinfusion samples. This has been examined in 3 animals to date. In these

experiments, 2 consecutive 10-min samples were collected immediately following LC activation. The results did not indicate that NA release was substantially greater in the first 10 min than in the 2nd 10 min. In these experiments, the second 10-min sample contained NA concentrations that were either quite similar to the first 10-min sample or were intermediate between the first 10-min sample and the recovery sample. These results suggest that releasable NA is not being rapidly depleted following LC activation. A second possible explanation for the ceiling effect is that enhanced NA release results in an activation of presynaptic α_2-NA receptors that inhibit NA release. This can be tested by examining the NA response to moderate- and high- level LC stimulation in the presence of an α_2-antagonist added to the dialysis perfusion buffer. These studies have been initiated (in collaboration with Dr. Ron Kuczenski) and substantial pilot data have been obtained. We have now resolved some initial technical difficulties with the microdialysis methods and are making rapid progress on this Specific Aim.

PILOT STUDIES

In addition to completing studies that were previously proposed, experiments relevant to the more general aims of the grant were completed as pilot or feasibility studies.

Involvement of the medial septal region in LC/NA modulation of forebrain EEG. The basal forebrain cholinergic nuclei, located in the substantia innominata/nucleus basalis of Meynert and the medial septal area/diagonal band of Broca are thought to play important roles in the modulation of cortical and hippocampal EEG. These nuclei receive a dense LC/NA innervation. We have initiated a series of experiments designed to determine whether LC/NA influences on forebrain EEG state are mediated by these nuclei. Initially, the effects of the β-agonist, isoproterenol (ISO), and the β-antagonist, timolol (TIM), infused into the medial septum on cortical and hippocampal EEG were examined in halothane-anesthetized rats. In order to perform a preliminary evaluation of this hypothesis, the following 3 questions were examined in approximately 60 rats: 1) What is the effect of ISO when infused into the medial septum under conditions in which slow-wave activity predominates in neocortex and hippocampus? 2) What is the effect of ISO infused outside the region of medial septum? 3) What is the effect of TIM infused bilaterally into the medial septum under conditions in which cortical and hippocampal EEG are activated? 4) What is the effect of TIM infused bilaterally into medial septum on peri-LC bethanechol-induced EEG activation? In these experiments, 26 ga. guide cannulae were implanted over left and right medial septum, penetrating cortex 1.5 mm at an angle of 4° to permit placement of a 33 ga. infusion needle into medial septum while avoiding damage to the superior sagittal sinus and fibers of passage that travel along the most medial aspect of the septal area. Infusions consisted of 100-150 nl of vehicle or drug, at a concentration of 25 ug/ul, infused over a 1-min period. Halothane was adjusted to permit the appropriate level of anesthesia, dummy infusion needles were placed into left and right cannulae and 30-45 min of baseline EEG was collected. 10 min prior to a medial septum infusion, the dummy needle was removed and a needle loaded with 2% Pontamine Sky Blue dye in phosphate buffer saline or drug dissolved in this solution was inserted.

Bilateral vehicle infusions into the medial septum had no obvious EEG effects. In contrast, 1-10 min following 100-150 nl of unilateral ISO,

hippocampal theta activity was substantially increased bilaterally and, in the majority of cases, there was a less robust but clear decrease in cortical slow-wave activity. The duration of these responses ranged from 20 min to greater than 60 min and could be reversed with TIM infusion. Identical volumes of ISO infused into the striatum a similar distance from the lateral ventricle had no EEG effects, indicating that these effects of ISO are not due to diffusion into the ventricular system and action at a distant site. Similarly, ISO had no effects on forebrain EEG when infused into the lateral septum or directly into the lateral ventricle approximately 1 mm posterior to the posterior end of medial septum, or into substantia innominata.

Under conditions in which forebrain EEG was in an activated state, unilateral TIM infusion had no EEG effects. In contrast, bilateral TIM resulted in a shift in hippocampal EEG from nearly pure-theta activity to mixed frequency activity and the appearance of large-amplitude, slow-wave activity in cortex.

The effects of TIM on peri-LC bethanechol-induced EEG activation were also examined. In these experiments, peri-LC infusions were made under 3 experimental conditions; prior to any septal infusions, 10 min following bilateral medial septal vehicle infusions, or 10 min following bilateral medial septal TIM infusions. Bilateral TIM blocked or severely attenuated the peri-LC bethanechol-induced activation of cortical and hippocampal EEG.

To summarize, as was observed with unilateral LC activation, unilateral infusions of the β-agonist, ISO, elicited bilateral EEG activation in hippocampus and cortex. Unilateral medial septal infusions of the β-antagonist, TIM, had no effect on either cortical or hippocampal EEG, whereas bilaterally infused TIM substantially decreased indices of EEG activation in both structures.

One possible explanation for the lack of effects of ISO when infused into substantia innominata on either cortical or hippocampal EEG is that, given the relatively large size of this area and the relatively small infusion volumes, the drug was not diffusing throughout an adequate volume of the structure to elicit EEG changes. Therefore, in an additional 4 cases, unilateral and bilateral ISO infusions were made in which the concentration of the drug was doubled and the infusion volume was either doubled (300 nl) or tripled (450 nl). These infusions had no obvious consistent effects on either hippocampal or cortical EEG.

Finally, in limited, preliminary experiments to assess whether the neocortical and hippocampal EEG activation observed in the halothane-anesthetized animal is atropine-sensitive, the effects of systemic atropine (75 mg/kg; ip) on spontaneous and tail-pinch-induced EEG were examined in 2 animals. This dose of atropine blocked the effects of locally infused bethanechol on LC discharge rates but did not have obvious effects on tail-pinch-induced or spontaneous cortical EEG desynchronization or hippocampal theta.

Assessment of monoamine release via microdialysis in unanesthetized monkey. We have performed microdialysis in an awake, chair-restrained cynomolgus monkey (Macaca fascicularis) to assess extracellular monoamine concentrations. This involved the development of techniques and equipment, as well as implementing procedures similar to those that would be used in proposed Specific Aim 3. Amphetamine administration was used in these experiments to demonstrate the specificity and sensitivity of the methods.

Because monkeys exhibit large individual differences in brain structure and size, and thus stereotaxic coordinates, for both cortical and subcortical structures, the initial step for this study was to perform an MRI brain scan on this animal to accurately determine the stereotaxic locations of target structures. With the head fixed in a plastic stereotaxic instrument, MRI scans

were performed in the 3 standard stereotaxic planes. The resulting images were subjected to a detailed analysis in order to determine the stereotaxic locations of target structures.

After a two-week recovery period, the monkey underwent surgery for the implantation of dialysis guide cannulae and a device for later immobilizing the head. Using aseptic techniques, guide cannulae were cemented in place bilaterally over: 1) primary motor cortex (n=4); 2) parietal cortex (n=4); and 3) the head of the caudate nucleus (n=4). The device that was later used to immobilize the head during chairing also served to hold a protective cap to prevent access by the animal to the cannulae and other parts of the head implant while he was in his home cage. After recovery from surgery, the monkey was habituated to the chairing procedure in daily sessions, including having the head fixed in position for 2-3 hours at a time. Following an additional 1-month period, collection of dialysis samples was initiated. 2-3 dialysis probes were inserted per session, and each session consisted of 4 days of repeated dialysis. On Day 1, the animal was chaired, the head fixed, dialysis probes inserted, the cap replaced, and the animal returned to its home cage. On Day 2, the animal was chaired, the head fixed, probes were connected to the perfusion pump, and sample collection was initiated. After 4 20-minute baseline samples were collected, amphetamine (0.25 mg/kg) was administered subcutaneously, and 3 additional samples were collected. Days 3 and 4 were identical to Day 2. At the end of Day 4, the probes were removed. Sessions were separated from each other by a 1-2 week period. After 5 such sessions, all possible sites had been used, some more than once. At this time, the animal was deeply anesthetized, dye was infused through dialysis probes reinserted through each of the guide cannulae, and the animal was perfused using our standard protocol for immunohistochemical experiments.

In caudate dialysis samples, DA (approx. 100 fmol/sample), DOPAC, HVA, 3MT and 5HIAA were reliably detectable. Amphetamine increased DA concentrations in caudate samples approximately 10-fold. In samples from parietal and motor cortices, DA (approx. 5 fmol/sample), NE (approx. 3 fmol/sample), 5HIAA, DOPAC, HVA and MHPG were all quantifiable. (Our current HPLC assays have limits of quantification, i. e., 3X noise, near 2 fmoles per sample.) Amphetamine increased both NA and DA concentrations approximately 10-fold. In all regions, HVA was present in much higher concentrations than DOPAC, a pattern opposite that observed in rodents. As expected, NA was not detectable in baseline or post-drug samples from caudate.

The results of our attempts to dialyze a single site over several days, or to reinsert dialysis probes into previously used sites, indicated that such an approach is not currently feasible. In both cases, basal dialysate DA, NA, and their metabolites were substantially decreased, in some cases below detection limits, and the responses to amphetamine challenge were significantly diminished, and in some cases no longer evident. We and others have observed similar changes following repeated probe insertions into rodent brain. For these reasons, the proposed studies will use each dialysis site only once.

Histological analyses revealed that the MRI procedure substantially enhanced the accuracy of guide-cannula placement, relative to a purely stereotaxic approach. Most of the probe sites were found to be within 0.5 mm of their intended locations.

To summarize, these results demonstrate the feasibility of applying microdialysis methodologies, and of measuring extracellular DA and NA and their catabolites in cortical and subcortical regions in awake monkey.

We are now preparing a second monkey for an additional dialysis experiment. This monkey has undergone an MRI scan and is scheduled for surgery in one month. The usefulness of the MRI scan has been enhanced by implanting fiduciary markers in a short surgery 10 days prior to the MRI scan. The markers are glass cylinders, 1mm in outside diameter, 3 mm in length, and filled with CuSO4 (0.05 M). They were implanted into the skull in holes drilled orthogonal to the skull surface at predetermined stereotaxic coordinates. The MRI scan will be used to determination the exact relationship between the fiduciary markers and particular sites, such as the central fissure. This will allow the placement of probes relative to the fiduciary marks, enhancing the accuracy of placement relative to particular anatomical structures.

Effects of LC activation on neuronal activity in somatosensory cortex [Publication 12] In these studies a recording/infusion probe was used to activate the neurons of the LC in a reversible and verifiable manner in halothane-anesthetized rats. Simultaneously, recordings were obtained from neurons in the hindlimb region of primary somatosensory cortex. These were activated by appropriate peripheral somatosensory stimuli (air puff or electrical stimulation delivered to the receptive field). Somatosensory responses and spontaneous discharge activity were recorded during baseline conditions, LC activation, and LC recovery. LC stimulation effects were highly replicable both within and between animals. Baseline somatosensory responses consisted of a brief, short-latency activation followed by a longer duration pause, in which activity decreased to below background levels, and a gradual return to prestimulus discharge rates. During LC activation, the brief initial response was somewhat reduced, but the previous long-latency reduction in activity became an extended activation. Overall, the absolute magnitude of the total response was increased. Since background activity was reduced during LC activation, the ratio of stimulus-elicited to background activity was considerably enhanced. Thus, the effect of LC stimulation was similar in many regards to that previously reported for iontophoretic application of NA to cortical sensory neurons, although certain differences were also evident. In the last year, the analyses of these data have been completed and a manuscript describing these results has been submitted for publication.

FULL-LENGTH PUBLICATIONS: YEARS 04-06

1. Swick, D., Pineda, J.A., and Foote, S.L. Effects of systemic clonidine on auditory event-related potentials in squirrel monkeys. Brain Res. Bull., in press.

2. Pineda, J.A. and Swick, D. Visual P3-like potentials in squirrel monkey: effects of a noradrenergic agonist. Brain Res. Bull. 28: 485-491, 1992.

3. Pineda, J.A., Swick, D., and Foote, S.L. Noradrenergic and cholinergic influences on the genesis of P3-like potentials. In: Event Related Potentials of the Brain (Suppl. 42 to Electroencephalography and Clinical Neurophysiology). Ed: C.H.M. Brunia. pp. 165-172, 1991.

4. Pineda, J.A. and Westerfield, M. Monkey P3 in an "oddball" paradigm: pharmacological support for multiple neural sources. Submitted.

5. Pineda, J. and Nava, C. Event-related potentials in macaque monkey during passive and attentional processing of faces in a priming paradigm. Beh. Brain Res., in press.

6. Pineda, J.A., Holmes, T.C., Swick, D., and Foote, S.L. Brainstem auditory evoked potentials in squirrel monkey (Saimiri sciureus). Electroenceph.

Clin. Neurophysiol. 73:532-543, 1989.

7. Pineda, J.A., Holmes, T.C., and Foote, S.L. Intensity-amplitude relationships in monkey event-related potentials: Parallels to human augmenting-reducing responses. Electroenceph. clin. Neurophysiol. 78:456-465, 1991.

8. Swick, D., Pineda, J.A., Schacher, S., and Foote, S.L. Locus coeruleus neuronal activity in awake monkeys: relationship to spontaneous EEG and auditory P300-like potentials. Submitted.

9. Berridge, C.W. and Foote, S.L. Effects of locus coeruleus activation on electroencephalographic activity in neocortex and hippocampus. J. Neurosci. 11:3135-3145, 1991.

10. Foote, S.L., Berridge, C.W., Adams, L.M. and Pineda, J.A. Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting, and attending. Prog. Brain Res. 88:521-532, 1991.

11. Berridge, C.W., Page, M.E., Valentino, R.J., and Foote, S.L. Effects of locus coeruleus inactivation on electroencephalographic activity in neocortex and hippocampus. Neuroscience 55:381-393, 1993.

12. Adams, L.M. and Foote, S.L. Effects of locus coeruleus activation on spontaneous and sensory-evoked activity of neocortical somatosensory neurons. Submitted.

13. Berridge, C.W., Arnsten, A.F.T., and Foote, S.L. Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological, and behavioral studies in animal models. Psychol. Med., in press. (Editorial)